Original article

Determination of eligibility to antiretroviral therapy in resource limited settings using total lymphocyte counts, hemoglobin and body mass index among HIV positive patients

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Abstract

Background: Acquired Immunodeficiency Syndrome is a serious public health problem in Ethiopia**.** $CD4^+$ T cell count testing is the standard method for determining eligibility for antiretroviral therapy. However, automation for $CD4^+$ T cell count is not widely available in sub-Saharan Africa including Ethiopia.

Objective: This study was to determine eligibility for antiretroviral therapy in resource-limited settings using total lymphocyte counts, hemoglobin and body mass index among HIV positive patients.

Materials and methods: $CD4^+$ T cell count was determined using Becton Dickinson FACS count analyzer. Total lymphocyte count and hemoglobin concentration were measured by a Cell Dyne 1800 hematology analyzer and body mass index was determined. Correlation of total lymphocyte count, hemoglobin and body mass index with $CD4^+$ T cell count was determined by Pearson's correlation coefficient and p-value.

Results: The correlation between $CD4^+$ T cell count and Total Lymphocyte Count (TLC) was not strong, but the association between $CD4^+$ T cell count and TLC was highly significant and correlation between $CD4^+$ T cell counts with hemoglobin were very weak. The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of TLC using threshold value of 1000 cells/mm^3 for $CD4^+$ T cell counts <350 cells/mm^3 were 3% , 94%, 17% and 71%, respectively. Total lymphocyte count threshold of 1750 cells/mm^3 were the better predictor of $CD4^+$ T cell counts of <350 cells/mm^3 when compared to < 200 cells/mm^3.

Conclusion: TLC showed weak correlation with $CD4^+$ T cell counts but the association between $CD4^+$ T cell count with TLC was significant ($p<0.0001$). The TLC threshold of 1750 cells/mm^3 were the most accurate predictors of $CD4^+$ T cell counts of <350 cells/mm^3. Therefore, the significant association of TLC with $CD4^+$ T cell count may suggest that TLC could be used as marker for $CD4^+$ T cell count in determining anti-retroviral treatment initiation when $CD4^+$ T cell count is not available particularly in rural settings where laboratory facilities are lacking. [*Ethiop. J. Health Dev.* 2013;27(1):48-54]

Introduction

Human immunodeficiency virus (HIV) is a member of the retroviruses that causes acquired immunodeficiency syndrome (AIDS), a condition in humans associated with progressive failure of the immune system (1, 2). Human immunodeficiency virus infection leads to a state of generalized immune activation which leads to increased T- cell turnover (production and destruction), increased activation-induced death of T- cells, a decline in the size of the $CD4^+$ T cell pool and a state of activation- induced immunodeficiency. The virus can also mutate and escape immune mediated opposition by suboptimal Cytotoxic T lymphocytes (CTL) followed by altered functions of the antigen presenting cell's (APCs) that may exhibit diminishing ability to elicit immune responses (3).

At the end of 2010, an estimated 34 million people were living with HIV worldwide. This reflects the continued growth in the number of new HIV infections (2.5 million) as well as deaths (2.1 million) that were reported in the same year (4). In sub-Saharan Africa, the estimated numbers of adults and children living with the virus at the end of 2010 were 22.5 million, nearly 70% of the global cases (4). Based on the 2007 single point estimate, the Government of Ethiopia (GOE) projected HIV prevalence to be 2.4% in 2010. The 2011 Ethiopia Demographic and Health Survey (EDHS) data showed that the urban adult HIV prevalence was 4.2 percent and rural adult HIV prevalence was 0.6 percent (5). In the Amhara National Regional State of Ethiopia, the prevalence of HIV among adults in 2010 was reported to be 2.7 % (6).

The WHO guidelines for ART initiation in low-income countries state that HIV-infected individuals should start ART when they have WHO stage IV disease, stage III disease and a $CD4^+$ T cell count of <350 cells/mm3, or stage I or II disease with $CD4^+$ T cell count <200 cells/mm3 (7). Recently WHO has recommended increasing this threshold for stages I and II to 350 cells/mm3 (8).

The proportion of ART patients that had CD4 count was 19.9% and 18.2% by the year 2003 in Ethiopia and the Amhara Region, respectively. Despite that, the proportion of ART patients that had CD4 count increased

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to 68.6% by the year 2005 in the country (9). Although $CD4^+$ T cell count is commonly used to determine ART initiation, the WHO guidelines recommend the use of clinical staging alone or in combination with total lymphocyte counts (TLCs) of < 1200 cells/mm3 in order to determine ART eligibility (7). However, many studies have found both clinical stages III/IV and the TLC threshold to have poor sensitivity for low $CD4^+$ T cell counts, leading researchers attempt to define other TLC thresholds which better correspond to $CD4^+$ T cell counts of < 200 or 350cells/mm3 (10-12).

Studies which have incorporated hemoglobin (Hgb) or haematocrit into clinical algorithms have shown improved performance of TLC in predicting low $CD4^+$ T cell counts (13-15). However, such studies have treated the $CD4^+$ T cell count threshold as an absolute standard, where initiating treatment in patients with $CD4^+$ T cell counts above 200 cells/mm3 is undesirable. As delaying ART until $CD4^+$ T cell counts fall below 200 cells/mm3 results in increased mortality (16) and most studies from sub-Saharan African settings have shown high mortality rates in the first year of therapy (17-19), it seems reasonable to adopt the more liberal ART eligibility criteria of <350 cells/mm3. The laboratory determination of the $CD4^+$ T cell count requires the use of a fluorescence-activated Cell-Sorter (FACS) flow-cytometer that makes the process both expensive and labor-intensive. In terms of comparative costs, a single $CD4^+$ T cell count may be 30–50 times more expensive than a complete blood cell count (20). Determining total lymphocyte count, hemoglobin concentration and BMI are inexpensive to measure and do not require advanced technology. However, there is no sufficient information concerning the correlation of TLC, Hgb and BMI with $CD4^+$ T cell counts in the study area in particular and in Ethiopia in general. Nevertheless, Abuye et al. reported that CD4 counts were independently associated with BMI among HIV-negative Ethiopians. They also concluded that low BMI among Ethiopians may have contributed to their overall low CD4 count (21).

The present study was designed to investigate the eligibility of antiretroviral therapy in resource-limited settings using total lymphocyte counts, hemoglobin and body mass index.

Methods

A hospital-based cross-sectional study was conducted at University of Gondar Hospital. At the time of the study the hospital gives pre-antiretroviral therapy to 3000 HIV positive patients. In this study, only adult patients under pre-ART follow up were included. However, pregnant women, smokers, patients with tuberculosis, malaria and acute viral infections were excluded. Using a pretested and structured check list, information on sex, age, marital status, level of education, occupation, TB, diarrhea, and WHO stages of HIV were assessed. The data were completed by face-to-face interview. Five milliliters of venous blood was collected from each study subject using disposable tubes for routine laboratory investigation. The left over samples were used for this study but a written informed consent was taken from each patient during collection of the socio-demographic information. Blood samples were collected under aseptic conditions between 8:00 and 10:00 am and analyzed within an hour by mixing with Ethylene Diamine-Tetra-Acetic Acid (EDTA) anticoagulant for five minutes. The hemoglobin concentration and total leukocyte count (TLC) were determined using Cell Dyne 1800 hematology analyzer (Abbott Cell Dyne Operators manual). From the same blood samples, a $CD4^+$ T cell count was enumerated using BD FACS count (BD Biosciences instrument user's guide) following the manufacturer's instructions.

All study subjects were assessed for malnutrition using body mass index (BMI) (22). The reliability of the study findings were guaranteed by implementing quality control (QC) measures throughout the whole process of the laboratory work. All materials, equipment and procedures were adequately controlled. FACS count (BD Biosciences instrument user's guide) and Cell Dyne 1800 (Abbott Cell Dyne Operators manual) were checked for linearity and validity by using quality control reagents. Pre-analytical, analytical and post-analytical stages of quality assurance that were incorporated in Standard Operating Procedures (SOPs) were strictly followed. Appropriate volume of blood and anticoagulant were used to maintain the specimen's quality. Every day before the samples were investigated, control reagents with low, medium and high concentration were simultaneously investigated by the Cell Dyne 1800 and FACS count machines to maintain the reagents quality.

The data was entered, cleaned and analyzed using SPSS statistical computer software version 20. Pearson's correlation coefficient, sensitivity, specificity, positive and negative predictive values and a Receiver Operating Characteristic (ROC) curve were used for statistical analysis to determine the association between TLC, hemoglobin concentration and BMI with that of $CD4^+$ T cell count. A p-value of less than 0.05 was considered as statistically significant. Ethical approval was obtained from the University of Gondar, School of Biomedical and Laboratory Sciences Ethical Clearance Committee before going ahead with the study. Each study participant was asked to participate voluntarily. When the study participants agreed to participate, written informed consent was obtained from each of them. Each study participant was informed about the objective of the study which was to contribute to the improvement and to look for alternative evaluation method to initiate anti-retrovirus treatment for poor patients in resource poor

health facilities. Voluntary participation and the right to withdraw at any time were emphasized.

Results

Socio-demographic Characteristics:

A total of 341 pre-ART HIV positive patients were recruited for the present study. One hundred twenty-three patients (36.1%) were males and 218 (63.9%) were females. The median age of the study participants was 30 years (IQR=26.5-38.5) and the majority (75.6 %) were living in urban settings. Three hundred eight (90.3%) of the participants were Orthodox Christians and data on marital status showed that 158 (46.3%) were married (Table 1).

Table 1: **Socio-demographic Characteristic of HIV Positive Study Participants at the University of Gondar Hospital, Northwest Ethiopia, 2012**

Characteristic		Male N (%)	Female N (%)	Total N (%)
Age (yrs)	18-29	31 (22)	107 (78)	138 (40.5)
	30-39	53 (41)	75 59)	128 (37.5)
	40-49	28 (58)	20 (42)	48 (14)
	≥50	11 (41)	16 (59)	27 (8)
Residence	Urban	89 (34.5)	169 (65.5)	258 (75.6)
	Rural	34 (40.9)	49 (59.1)	83 (24.4)
Marital status	Single	34 (66.7)	17 (33.3)	51 (15)
	Married	64 (40.5)	94 (59.5)	158 (46.3)
	Divorced	22 (24.7)	67 (75.3)	89 (26.1)
	Widowed	2 (5.9)	32 (94.1)	34 (10)
	Separated	1 (11.1)	8 (88.9)	9 (2.6)
Religion	Orthodox	112 (36.4)	196 (63.6)	308 (90.3)
	Muslim	10 (37.1)	17 (62.9)	27 (7.9)
	Protestant	0	2 (100)	2 (0.6)
	Catholic	1 (25)	3 (75)	4 (1.2)
Occupation	Merchant	19 (35.2)	35 (64.8)	54 (16)
	Civil servant	29 (54.7)	24 (44.3)	53 (15.5)
	House wife	0	61 (100)	61 (17.9)
	Student	2 (13.3)	13 (86.7)	15 (4.4)
	Farmer	15 (53.5)	13 (46.5)	28 (8.2)
	Driver	9 (100)	0	9 (2.6)
	Daily laborer	38 (41.7)	53 (58.3)	91 (26.6)
	Private	11 (40.9)	19 (59.1)	30 (8.8)
Educational status	Illiterate	25 (23.8)	80 (76.2)	105 (30.8)
	Elementary school	48 (44)	61 (56)	109 (32)
	High school	31 (36)	55 (64)	86 (25.2)
	Certificate	1 (10)	9 (90)	10 (2.9)
	Diploma	14 (56)	11 (44)	25 (7.1)
	Degree and above	4 (66.7)	2 (33.3)	6 (2)
Total		123 (36.1)	218 (63.9)	341(100)

Immuno-hematological Profiles of the Pre-ART HIV Positive Patients:

The median $CD4^+$ T cell count and total lymphocyte count (TLC) were 342 cells/mm^3 (IQR=212-496) and 2000 cells/mm^3 (IQR=1500-2600), respectively. The median hemoglobin (Hgb) concentration and body mass index (BMI) were 13.1 g/dl (IQR=11.6-14.1) and 20.1 kg/m^2 (IQR=18.3-22.6), respectively (Table 2).

In this study, 83 (24.3%) of the study participants had $CD4^+$ T cell counts of <200 cells/mm^3, 96 (28.2%) of them had $CD4^+$ T cell counts between 200 to 350 cells/mm^3 and the rest (47.5%) had > 350 cell/mm^3. Forty eight (14.1%) of the study participants had total lymphocyte counts (TLC) of <1200 cells/mm^3. On the other hand, 103 (30.2%) study participants had hemoglobin concentrations of <12g/dl and 91(26.7%) of them had body mass index of <18.5 kg/ m^2. Fifty two (62.7%) participants had total lymphocyte counts of >1200 cells/mm^3 with $CD4^+$ T cell counts of <200 cells/mm^3 (Table 3).

Correlation of Total Lymphocyte Count, Hemoglobin and Body Mass Index with $CD4^+$ T Cell Count:

Correlation between $CD4^+$ T cell count and TLC (n=341) (r=0.425) was not strong, but the association between

Table 2: **Immuno-hematological profiles and body mass index of the study participants at University of Gondar Hospital, Northwest Ethiopia, 2012**

	N	Minimum	Maximum	Median	IQR	Mean	St Dev.
Hemoglobin	341	5.80	18.30	13.1	11.6-14.1	12.8	1.9
Total lymphocyte counts	341	500.0	5220.00	2000	1500-2600	2127.6	908.4
$CD4^+$ T cell counts	341	13.00	1103.00	342	212-496	367.4	214.2
Body mass Index	341	12.32	33.60	20.1	18.3-22.6	20.6	3.4

IQR-Inter quartile range

$CD4^+$ T cell count and TLC was significant ($p<0.0001$)and correlation between $CD4^+$ T cell count with hemoglobin ($r=0.188$, $p=0.0005$) was very weak. Moreover, there was no significant correlation between BMI and $CD4^+$ T cell count. The Positive Predictive Value (PPV) for $CD4^+$ T cell count of <200 cells/mm^3 using a threshold value of 1000 cells/mm^3 for TLC was 78%, while the sensitivity was only 17%. The Negative Predictive Value (NPV) was 79%, with 98% specificity. The sensitivity, specificity, PPV and NPV of TLC using a threshold value of 1000 cells/mm^3 for $CD4^+$ T cell count of <350 cells/mm^3 were 3% , 94%, 17% and 71%, respectively (Table 4). A TLC threshold value of 1200 cells/mm^3 with $CD4^+$ T cell count of <200 cells/mm^3 gave a sensitivity of 25% and a specificity of 97%. A TLC threshold of 1750 cells/mm^3produced sensitivity, specificity, PPV, and NPV of 65%, 69%, 4%, and 86%, respectively with an accuracy of 79% for $CD4^+$ T cell count of <200 cells/mm^3. Initiation of ART to all HIV positive participants with a TLCs of ≤ 2250 cells/mm^3 produced a sensitivity of 82% for $CD4^+$ T cell count of <200 cells/mm^3 (Table 4).

Table 3: **Distribution of Immuno-hematological profiles and Body mass index of study participants by $CD4^+$ T cell count strata at University of Gondar Hospital, Northwest Ethiopia, 2012**

		$CD4^+$ T cell count/mm^3			Total
		≤200 N (%)	200-350 N (%)	>350 N (%)	N (%)
TLC/mm^3	≤1200	29(34.9)	15(15.6)	4(14.1)	48(14.1)
	1200-3500	52(62.7)	77(80.2)	138(85.2)	267(78.3)
	>3500	2(2.4)	4(4.2)	20(12.3)	26(7.6)
Hgb g/dl	≤12	39(47.0)	23(24.0)	41(25.3)	103(30.2)
	12-18	44(53.0)	72(75)	121(74.7)	237(69.1)
	>18	0	1(1)	0	1(0.3)
BMI Kg/m^2	<18.5	32(38.6)	27(28.1)	32(19.8)	91(26.7)
	18.5-25	49(59.0)	60(6.5)	105(64.8)	214(62.8)
	>25	2(2.4)	9(9.4)	25(15.4)	36(10.6)
Total		83(24.3)	96(28.2)	162(47.5)	341(100)

Table 4: **Sensitivity, specificity and predictive values of TLCs alone or in combination with Hgb and BMI in predicting $CD4^+$ T cell counts ≤200cells/mm^3 and ≤350cells/mm^3 at University of Gondar Hospital, Northwest Ethiopia, 2012**

TLC LTD	TLC UTD	Hgb TD	BMI TD	$CD4^+$ T cell count ≤200cells/mm^3					$CD4^+$ T cell count ≤350cells/mm^3					
				Sen.	Sp	PPV	NVP	ACC	Sen.	SP	PPV	NVP	ACC	FP
CD4 alone				1.00	1.00	1.00	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0
TLC alone														
1000				0.17	0.98	0.78	0.79	0.79	0.03	0.94	0.17	0.71	0.68	0.02
1200				0.25	0.97	0.72	0.80	0.79	0.08	0.91	0.21	0.75	0.67	0.04
1500				0.48	0.84	0.49	0.83	0.75	0.28	0.78	0.33	0.73	0.64	0.16
1750				**0.65**	**0.69**	**0.4**	**0.86**	**0.79**	**0.50**	**0.65**	**0.36**	**0.77**	**0.61**	**0.31**
2000				0.74	0.58	0.36	0.87	0.62	0.64	0.56	0.36	0.80	0.58	0.42
2250				0.82	0.43	0.32	0.88	0.52	0.78	0.43	0.35	0.83	0.53	0.57
2500				0.87	0.36	0.30	0.89	0.48	0.86	0.37	0.35	0.87	0.51	0.64
2750				0.92	0.24	0.28	0.90	0.70	0.91	0.25	0.32	0.87	0.43	0.76
3000				0.93	0.18	0.02	0.89	0.36	0.92	0.18	0.31	0.85	0.39	0.82
3500				0.96	0.11	0.26	0.90	0.31	0.95	0.10	0.29	0.83	0.34	0.90
Hemoglobin alone														
		12		0.47	0.28	0.38	0.77	0.70	0.28	0.81	0.70	0.41	0.60	0.19
TLC combined with Hgb and/or BMIs														
1750	2750	11	18	0.04	0.97	0.30	0.76	0.75	0.04	0.98	0.4	0.72	0.71	0.03
1750	2750	12	18	0.05	0.97	0.31	0.76	0.74	0.04	0.96	0.31	0.72	0.70	0.05
1750	2750	11		0.05	0.96	0.33	0.72	0.70	0.05	0.96	0.27	0.76	0.74	0.04
1750	2750	12		0.08	0.92	0.24	0.76	0.71	0.08	0.91	0.28	0.72	0.68	0.09
2000	3000	11		0.01	0.96	0.09	0.75	0.73	0.04	0.97	0.36	0.72	0.70	0.04
2000	3000	12		0.04	0.92	0.13	0.75	0.71	0.05	0.93	0.22	0.71	0.68	0.08
2000	3000		18	0.06	0.93	0.22	0.76	0.72	0.08	0.94	0.35	0.72	0.70	0.07
2000	3000	11	18	0.04	0.99	0.50	0.76	0.76	0.03	0.99	0.50	0.72	0.72	0.01

NB: Body Mass Index=Weight (Kg)/Height (m^2), Sen. -Sensitivity, SP =Specificity,
FP =false positive, PPV- Positive Predictive Value, NPV-Negative Predictive Value, UTD-Upper Threshold, LTD-Lower Threshold, TD-Threshold, ACC-Accuracy

Discussion

The peripheral blood absolute CD4+ T cell count and CD4+ T cell proportion are among the surrogate markers for the assessment of the risk for progression to AIDS in HIV-infected individuals. They are clinically useful in assessing the risk of developing certain AIDS-related opportunistic infections and for timing the initiation of antiretroviral and prophylactic antimicrobial therapies (23). The median $CD4^+$ T cell count and the total leukocyte count observed among HIV-positive patients in Gondar (342 cell/mm^3 and 2000 cell/mm^3) were relatively similar to the reports of a similar study from Uganda, which were 239 cells/mm^3 and 1830 cells/mm^3, respectively (24). One of the possible reasons for the difference between the findings of these two studies could be the delay in time during laboratory investigations. However, the median CD4+ T cell count in the present study (342 cells/mm^3) was lower than the finding in the study from Brazil (430 cells/mm^3), but the median TLC (1900 cells/mm^3) was almost comparable (22). On the other hand, lymphocyte counts reported for adults from the Central African Republic were significantly higher than those found in studies conducted in Ethiopia (25). The difference and/or discrepancies observed on $CD4^+$ T cell count among different population groups could be because of the different ethnic and socioeconomic factors that can influence difference in immune-hematological profiles.

The proportion of HIV positive patients that had $CD4^+$ T cell counts of <200 cells/mm^3 and $CD4^+$ T cell counts of <350 cells/mm^3 in the present study were 24.3% and 52.5%, respectively. This was by far different from a similar report from Indonesia, where 66% had $CD4^+$ T cell counts of <200 cells/mm^3 and 81% had <350 cells/mm^3 (26). However, in the present study, tuberculosis patients, pregnant mothers, WHO clinical stage IV patients and smokers, were excluded. Previously**,** Irwin had reported low CD4+ T-cell counts associated with a variety of conditions, including many viral infections, bacterial infections, parasitic infections, sepsis, tuberculosis, coccidioidomycosis, burns, trauma, and intravenous injections of foreign proteins, malnutrition, over-exercising, pregnancy, normal daily variation, psychological stress, and social isolation (27-29).

In the present study, $CD4^+$ T cell counts were slightly correlated with TLC and the association was strong and significant (<0.0001). Recently, Githinji et al. from Kenya reported that TLC and CD4 counts were positively correlated in children with severe immuno-suppression because of HIV-1 infection (30). In addition, according to reports from China the correlation between $CD4^+$ T cell counts with TLC was found to be statistically significant (31). Strong correlation between $CD4^+$ T cell counts and TLC was also reported in India (32), Indonesia (26), Iran (33) and England (34). However, Daka and Loha from the Southern Ethiopia reported low sensitivity and specificity of TLC as a surrogate measure of CD4 counts (35). Low correlation between $CD4^+$ T cell counts with hemoglobin was found in the present study. Correlation of hemoglobin concentration with CD4+ T cell count was not also significant in a study from Iran and China (33, 36). Correlation between $CD4^+$ T cell counts with BMI was not also statistically significant in the present study. This was also supported by other similar studies in Uganda and Indonesia that reported no significant correlation between $CD4^+$ T cell count and BMI (24, 26). However, it is believed that malnutrition reduces body weight, depletes energy stores, brings about loss of somatic protein (low muscle mass) and low levels of serum albumin, transfer in, pre-albumin and other visceral proteins (37). Nutritional deficiency has a major impact on immune function which may result in depression of lymphocyte count or function that are not desirable in an individual fighting invasive infection.

The WHO guidelines for ART initiation in low-income countries state that HIV-infected individuals should start ART when TLC is ≤1200 cells/mm^3 or $CD4^+$ T cell count is <200 cells/mm^3 (8). In the present study, pre-ART HIV positive study participants with $CD4^+$ T cell count of <200 cells/mm^3 that had TLCs of ≤1200 cells/mm^3 demonstrated a sensitivity of 25%; specificity=97%; PPV=72% and NPV=80%. Previous reports from India showed that pre-ART HIV positive study subjects having TLC of ≤1200 cells/mm^3 had a sensitivity of 70.3%, specificity of 95%, PPV of 89.7% and NPV of 84.9% for predicting $CD4^+$ T cell count of <200 cells/mm^3 (26). This finding indicated that taking similar $CD4^+$ T cell count and TLC among different population groups resulted in different sensitivity, specificity, positive predictive value and negative predictive values for initiating anti-retroviral therapy among HIV positive patients. The specificity, PPV and NPV of the present study were comparable with other studies, even though the sensitivity was found to be lower. The difference in sensitivity could be attributed to differences in factors related to: immuno-hematology, socioeconomic, biological, feeding habit, and even the virus species that caused the disease within the different population groups. HIV-1 is more virulent and more infective than HIV-2 (38). HIV-1 subtype C virus was reported to be the main strain in HIV infection in Ethiopia as well as in Eeastern and Southern Africa (37).

Eligibility for ART to all participants with a TLCs of ≤ 1750 cells/mm^3 produced the most accurate predictor of $CD4^+$ T cell count of <200 cells/mm^3 giving an accuracy of 79% (95% CI: 69-80). On the other hand treatment initiation for all patients with a TLC of <2000 cells/ mm^3, excluded all patients with a TLC of >3000 cells/mm^3.Threrefore, the use of hemoglobin concentration and BMI values for those patients with TLC of between 2000 cells/mm^3 and 3000cells/mm^3 might help determine eligibility for ART. In a study conducted in Uganda, Moore et al. reported that treatment algorithms with a TLC of < 2000 cells/mm^3

excluded all patients with a TLC of > 3000 cells/mm^3. They used hemoglobin and/or BMI values to determine eligibility for those with TLC values between 2000 to 3000 cells/mm^3 that marginally improved accuracy (23). Moreover, Gautam et al. (24) showed that low hemoglobin concentration values and low BMI are independent risk factors for HIV disease progression. Therefore, we suggest that incorporating hemoglobin concentration and BMI into the ART eligibility criteria could be valuable but it requires additional studies. Moreover, the present study demonstrated that using a TLC of 2250 cells/mm^3 to determine ART eligibility in subjects with WHO clinical stages I, II or III, could identify 82% of subjects with CD4$^+$ T cell count of <200 cells/mm^3, while 57% of subjects eligible for ART would have CD4$^+$ T cell counts of > 200 cells/mm^3. This is supported by other studies reporting that offering ART to all subjects with TLC of less than 2250 cells/mm3 produced a sensitivity of 88% (23).

The laboratory investigation method used in this study was only CD4$^+$ T cell count which is not the only perfect predictor of the severity of viral infection among HIV positive patients. The gold standards for determining eligibility for ART are both determining viral load, which measures the severity of viral infection, and CD4$^+$ T cell count. The proportion of HIV positive study subjects with CD4$^+$ T cell counts of < 200 cells/mm^3 and < 350 cells/mm^3 in the present study was not very high: 83 (24.3%) and 179 (52.5%), respectively resulting in a large number of subjects being not ART-eligible: 162 (47.5%).In conclusion, although the TLC showed weak correlation with CD4$^+$ T cell counts, the association between CD4$^+$ T cell count and TLC was significant ($p<0.0001$). The correlation between Hgb and CD4$^+$ T cell counts was weaker and there was no correlation between BMI and CD4$^+$ T cell counts. Eligibility for ART of all participants with TLCs of ≤ 1750cells/mm^3 produced the most accurate predictor of CD4$^+$ T cell count of <350 cells/mm^3 giving an accuracy of 79% and initiation of ART to all HIV positive participants with a TLCs of ≤ 2250 cells/mm^3 produced a sensitivity of 82% for CD4$^+$ T cell counts of <200 cells/mm^3. Therefore, although weak, the significant association of TLC with CD4+ T cell count may suggest the possibility that TLC could be used as marker for CD4$^+$ T cell count in determining anti-retroviral treatment initiation when CD4$^+$T cell count is not available particularly in rural settings where laboratory facilities are lacking. Further studies are also suggested to confirm the relationships using larger sample size.

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